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Abstract: Human psychological stress is the major environmental risk factor for major depression and certain of the anxiety disorders. Psychological stressors often occur in the context of the adult social environment, and they or the memory formed of them impact on the individual across an extended period, thereby constituting chronic psychosocial stress (CPS). Psychosocial stressors often involve loss to the individual, such as the ending of a social relationship or the onset of interpersonal conflict leading to loss of social control and predictability. Given the difficulty in studying the etio-pathophysiological processes mediating between CPS and brain and behavior pathologies in human, considerable effort has been undertaken to study manipulations of the social environment that constitute adulthood chronic psychosocial stressors in other mammals. The majority of such research has been conducted in rodents; the focus for a considerable time period was on rats and more recently both rats and mice have been investigated, the latter species in particular providing the opportunity for essential gene x chronic psychosocial stressor interaction studies. Key studies in the tree shrew demonstrate that this approach should not be limited to rodents, however. The animal adult CPS paradigms are based on resident-intruder confrontations. These are typified by the intruder-subject's brief proximate interactions with and attacks by, and otherwise continuous distal exposure to, the resident stressor. In contrast to humans where cognitive capacities are such that the stressor pertains in its physical absence, the periods of continuous distal exposure are apparently essential in these species. Whilst the focus of this review is on the stressor rather than the stress response, we also describe some of the depression- and anxiety disorder-relevant effects on behavior, physiology and brain structure-function of chronic psychosocial stressors, as well as evidence for the predictive validity of such models in terms of chronic antidepressant efficacy. Nonetheless, there are limitations in the methods used to date, most importantly the current emphasis on studying CPS in males, despite the much higher disorder prevalence in women compared to men. Future studies will need to address these limitations.

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Chronic psychosocial stressors in adulthood: studies in mice, rats and tree shrews

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Abstract

Human psychological stress is the major environmental risk factor for major depression and certain of the anxiety disorders. Psychological stressors often occur in the context of the adult social environment, and they or the memory formed of them impact on the individual across an extended period, thereby constituting chronic psychosocial stress (CPS). Psychosocial stressors often involve loss to the individual, such as the ending of a social relationship or the onset of interpersonal conflict leading to loss of social control and predictability. Given the difficulty in studying the etio-pathophysiological processes mediating between CPS and brain and behavior pathologies in human, considerable effort has been undertaken to study manipulations of the social environment that constitute adulthood chronic psychosocial stressors in other mammals. The majority of such research has been conducted in rodents; the focus for a considerable time period was on rats and more recently both rats and mice have been investigated, the latter species in particular providing the opportunity for essential gene x chronic psychosocial stressor interaction studies. Key studies in the tree shrew demonstrate that this approach should not be limited to rodents, however. The animal adult CPS paradigms are based on resident-intruder confrontations. These are typified by the intruder-subject's brief proximate interactions and attacks by, and otherwise continuous distal exposure to, the resident stressor. In contrast to humans where cognitive capacities are such that the stressor pertains in its physical absence, the periods of continuous distal exposure are apparently essential in these species. Whilst the focus of this review is on the stressor rather than the stress response, we also describe some of the depression- and anxiety-disorder relevant effects on behavior, physiology and brain structure-function of chronic psychosocial stressors, as well as evidence for the predictive validity of such models in terms of chronic antidepressant efficacy. Nonetheless, there are limitations in the methods used to date, most importantly the current emphasis on studying CPS in males, despite the much higher disorder prevalence in women compared to men. Future studies will need to address these limitations.

Keywords: social stressor; resident-intruder; control; depression; antidepressant; animal model

1. Introduction

For more than four centuries, physicians have reported on the association between adverse life events and psychopathology (e.g. for review see Rosen, 1959; Troisi, 2001). Today, epidemiological data are consistent with the view that stress is the most common risk factor for the development of mood and anxiety disorders, such as major depressive disorder (MDD) and generalized anxiety disorder (APA, 2013). More specifically, the diathesis-stress model proposes that traits regulated by the genome determine individual responsiveness to life-event stressors (Caspi and Moffitt, 2006; Pryce and Klaus, 2013; Risch et al., 2009). Of course, it constitutes a substantial challenge to study adverse life events that occur in the contexts of, for example, employment, finance, housing, health and social relationships, and to then identify which of these events are relevant to psychopathology and what their salient features are (Agid et al., 2000; Monroe and Reid, 2008). In addition to adverse life events during development, those occurring across adulthood are also associated with increased vulnerability to and the triggering of MDD (Brown et al., 1995; Kendler and Gardner, 2010; Kendler et al., 2002; Paykel, 2001). The adverse life events identified as etiological in one major MDD study were: death of a loved one, ending of a romantic relationship, personal failure or abandoned goal, chronic stress due to e.g. work, finances, legal problems, own health problems or interpersonal conflict, and distress over future events (Keller et al., 2007). It is proposed that the most relevant life events involve threat, loss, or humiliation, as directly experienced by the person (Brown et al., 1995; Monroe and Reid, 2008). When such an event is associated with MDD, it typically occurs 3-6 months prior to disorder onset. A further characteristic of adverse life events that are risk factors for MDD is that they are uncontrollable. In one large study, life events that involved major loss, humiliation or entrapment were predictive of MDD and, whilst it is not possible to independently assess its exact contribution, uncontrollability is certainly a major characteristic of these life-event dimensions (Kendler et al., 2003). In the human research laboratory, it is possible to independently manipulate the (un)controllability of aversive events, and such experiments have demonstrated that uncontrollability *per se* is a major determinant of behavior: it leads to reduced attempts to control the aversive stimulus and increased feelings of helplessness, particularly in MDD patients (Diener et al., 2008); this is referred to as the learned helplessness effect/state (Pryce et al., 2011; Seligman et al., 1971).

Therefore, adverse social life events can be of marked importance to individual well-being. The Australian zoologist Samuel A. Barnett, who coined the term social stress, and James P. Henry, the American physician, can be considered as founders of stress research in naturalistic settings, proposing similar concepts in humans and other animal species (Barnett, 1958; Barnett, 1964; Henry and Stephens, 1977). Taking a biological viewpoint, Barnett and Henry proposed that the social environment is a considerable source of stress and that the two processes of *fighting for control* and

losing control are of central importance to the organism's psychosocial situation and state (Henry and Stephens, 1977). In humans, loss of social rank, status and/or control are examples of the general class of loss-events which, as noted above, is increasingly recognized as a characteristic of risk factors for MDD (Brown, 1993; Keller et al., 2007). Clearly, the social group is a dominant feature of the environment in the majority of animal species, and a major function of social structure, relationships and interactions is to minimize social stress. Nonetheless, social stress is a characteristics of conspecific relationships and interactions, and of course also of inter-species predator-prey associations (Hinde, 1976; Sapolsky, 2005).

The stress hypothesis of mood and anxiety disorders has stimulated the development of a number of experimental manipulations of the environment in animals, with the aim of causing changes in behavior and brain that have relevance to stress-related psychopathologies in humans, i.e. animal models of relevance to mood and anxiety disorders (for recent reviews see e.g. Nestler and Hyman, 2010; Pryce and Seifritz, 2011; Slattery and Cryan, 2014). A number of the manipulations involve exposure to physical events, such as restraint, food or water deprivation, reversal of light-dark cycle, placement in water, and cage tilting. In the case of the most widely-used stressor, chronic unpredictable mild stress, several such physical events are presented on an unpredictable schedule, 1-2 per day, across a number of consecutive weeks (Willner, 1997; see Willner this volume). In addition, manipulations of the social environment have also been investigated and demonstrated to induce changes in behavior, brain and peripheral physiology. These studies have been conducted primarily in rodents. The most-studied psychosocial stressors are based on the resident-intruder paradigm. This uses social conflict between conspecifics to generate emotional stress. Classically in this experimental setting, one adult male, the intruder, is transferred into the home cage of another adult male, the resident, typically resulting in a fight where the resident is the "winner" and the intruder is the defeated stressed "loser". If the procedure is conducted on a single occasion it is regarded as an acute psychosocial stressor; if the intruder is placed with the resident repeatedly across a period of days or weeks then it is regarded as a chronic psychosocial stressor. In such repeated/chronic paradigms the intruder is transferred to an environment where it is still exposed to distal sensory signals, including threats, emitted from the resident but without experiencing physical attack. Koolhaas et al. (1997a) proposed that such social defeat is distinguished from other stressor paradigms with respect to the nature and the magnitude of the stress response. Moreover, it should be emphasized that social defeat induces changes in a variety of bio-physiological parameters each of which may have different temporal dynamics (Koolhaas et al., 1997b). For a detailed discussion of social stressor models in rodents see Koolhaas et al. (this volume).

In the present paper we review some of the resident-intruder manipulations that have been applied to induce MDD- and anxiety disorder-relevant behavioral, neurobiological and physiological

states in three mammalian species: the crepuscular-nocturnal, territorial and hierarchical mouse, the crepuscular-nocturnal, group-living and hierarchical rat, the most widely used species in preclinical stress research, and the diurnal, solitary non-rodent, the tree shrew. We describe the main characteristics of these manipulations that define them as psychosocial stressors, and discuss their specific features, advantages and limitations. The rationale for focusing on these species is that we have direct experience of studying adult chronic psychosocial stressors in them (Pryce: mouse; Fuchs: rat, tree shrew); the stressors that we have used are also given most attention, although comparisons are made with some of the other established paradigms.

2. Psychosocial stressors in mice

2.1 Resident-intruder paradigms

Adult male resident mice attack unfamiliar male conspecifics in their own territory. The classical resident-intruder test was designed based on observation of this aggressive behavior in both wild populations and laboratory strains (Benus et al., 1991; Parmigiani et al., 1998; Miczek et al., 2001). The resident-intruder paradigm can be applied to investigate the behavioral, physiological and neurobiological consequences of single or repeated social defeats. A review of methodological issues is provided in Bartolomucci et al. (2009). Of most relevance to the life events that are associated with psychopathology in humans are paradigms of repeated social defeat. The first of these, the sensory contact model, was developed by Kudryavtseva, Bakshtanovskaya and colleagues e.g. (Kudryavtseva et al., 1991). This paradigm comprises continuous distal sensory communication between a submissive C57BL/6 male intruder and a dominant, aggressive C57BL/6 resident, separated by a transparent, perforated divider, and 10 min daily proximate confrontations. For the intruder mouse, the identity of the resident mouse and its home cage/territory are different each day, and the duration of the stressor is 20 consecutive days. The behavior of intruder mice changes qualitatively across days: on earlier defeat days they exhibit active defence including withdrawal and sideways or upright postures, whilst on later defeat days they frequently exhibit passive defence (freezing or lying on the back) and are unresponsive to the resident's approaches, investigation and grooming (Kudryavtseva et al., 1991). A paradigm developed by Bartolomucci et al. (2001; 2004) also uses one mouse strain only, although in this case it is males of the CD-1 strain. A further change from the social contact paradigm is that the resident-intruder pairs remain fixed throughout the duration of the stressor, a feature taken on from the psychosocial stress paradigm developed in tree shrews (see section 4). Resident mice are housed individually for one week to allow for the establishment of an individual territory. Each such resident receives an intruder mouse (taken from group housing) and the two mice are allowed to interact freely for 10 min. After the interaction, they are separated by a perforated partition, and the partition is removed for 10 min on each of the 21 days of the paradigm.

In a third paradigm using the approach of distal sensory contact and daily proximate confrontation, two mouse strains are used; the resident dominants are ex-breeder, aggressive male CD-1 mice, and the subordinate intruders are male C57BL/6 mice. As in the paradigm of Kudryavtseva and Bakshtanovskaya, the intruder is placed in the cage of a different resident each day, and for 10 min (sometimes 5 min) per day. This paradigm, which has a duration of 10 days, is referred to as chronic social defeat (CSD) (Berton et al., 2006; Golden et al., 2011). A direct comparison of these different stressors, including of important differences such as whether stress effects are greater when the resident and experimental mice are from the same or different strains, remains to be conducted.

2.2 The nature of the stressor

The rationale underlying the above paradigms is that the combination of social defeat combined with continuous distal exposure to the resident in its territory constitutes a continuous (chronic) psychosocial stressor. One prediction from this is that conducting the daily confrontation and then removing the intruder to a neutral territory would be a less potent stressor, but to our knowledge this comparison has not been conducted. With regards to the psychological state induced in intruder mice, that physical attack persists despite the emitting of submissive behavior can be interpreted as the experiencing of the loss of social controllability. Support for this interpretation was provided by a study in which Pryce and colleagues studied CSD mice in a two-way active avoidance test with footshock; such mice exhibited an avoidance-escape deficit similar to that demonstrated by mice that had experienced prior exposure to inescapable footshock; experiencing a lack of social control appeared to generalize to a deficit in controlling a physical stressor (Azzinnari et al., 2014).

Another major factor to consider is the physical bite injuries, and subsequent physical pain stress, that occur during the attacks. As explained by Golden et al. (2011), wounding is a concern with the standard CSD protocol. It is proposed that CSD should be run with 20 or more resident-intruder pairs simultaneously, such that it is not possible to observe individual bouts of attack, and for a fixed duration of 10 min (Golden et al., 2011). Under these conditions, repeated across 10 days, biting that breaks the skin can be expected to occur frequently and there is also a risk of deep bite wounds; indeed, our initial studies using the standard protocol demonstrated this to be the case (Pryce, personal observation). For scientific, as well as ethical reasons, refinement was desirable: removal of wounded mice from the experiment could bias the sample in favor of mice that are effective at avoiding wounding; furthermore, continued inclusion of wounded mice is highly problematic to the study of etio-pathophysiology, particularly when immune-inflammation is of interest as a mechanism mediating between psychosocial stress and psychopathology, e.g. (Fuertig et al., 2016; Hodes et al., 2014). Accordingly, a refined CSD protocol was developed: resident-intruder pairs are run two at a time, and the duration of attacks is timed accurately and limited to a maximum of 1 min per day. The

lower incisor teeth of resident CD-1 mice are trimmed back every third day, to markedly reduce surface wounds and completely eliminate deep bite wounds (Azzinnari et al., 2014).

2.3 Stressor efficacy

Whilst the present paper focuses on the stressor rather than its consequences (behavioral, physiological, neurobiological), the efficacy, and therefore relevance, of psychosocial stressors can only be assessed in terms of these consequences, of course. The sensory contact paradigm resulted in consistent reductions in motor activity, as measured in the open field and forced swim test (Kudryavtseva et al., 1991). In the standard CSD paradigm, a major feature is that, immediately following the 10-day stressor, stressed mice are screened behaviorally in terms of whether or not they passively avoid an adult male mouse of the CD-1 strain i.e. the exact social stimulus by which they have been defeated during the previous 10 days (e.g. Golden et al., 2011; Krishnan et al., 2007; although see Berton et al., 2006). Mice that exhibit high passive avoidance relative to control mice (about 70%) are categorized as susceptible and mice that exhibit low passive avoidance similar to control mice (30%) are categorized as resilient. It is of course difficult to argue that the combination of CSD and subsequent passive avoidance of the same dominant stressor is a model of psychopathology, given that the avoidance behavior is adaptive (Russo et al., 2012). However, it should be noted that susceptible mice also exhibit reduced preference for gustatory reward in a two-bottle sucrose versus water test relative to controls whereas resilient mice do not (Krishnan et al., 2007). In line with the approach taken with other stressors (e.g. chronic unpredictable mild stress), with our refined CSD protocol we use an “inclusive” experimental design rather than screening mice into susceptible versus resilient sub-groups. Such CSD mice exhibit, relative to controls, consistent increases in Pavlovian fear learning, learned helplessness and physical fatigue (Azzinnari et al., 2014; Fuertig et al., 2016), decreases in effortful motivation for reward and in cognitive decision making to obtain reward in operant tests (Bergamini et al., 2016), increases in the levels of inflammatory markers in blood and brain (Azzinnari et al., 2014; Fuertig et al., 2016), changes in transcriptome expression in amygdala and prefrontal cortex (Azzinnari et al., 2014), and changes in brain functional connectivity (Grandjean et al., 2016). The use of an inclusive experimental design does not necessarily mean that the stressor efficacy is greater than in the susceptible-resilient design, at least when the majority of mice are susceptible in the latter.

One factor likely to be critical to the efficacy of any paradigm of mouse psychosocial stress is its chronicity. As noted above, this varies from 20 days in the sensory contact paradigm and 21 days in the stable resident-intruder paradigm, to only 10 days in the standard CSD paradigm. In addition to the other refinements described above, we increased the CSD paradigm to 15 days (Azzinnari et al., 2014). Chronic unpredictable mild stress is run for a duration of 21 days minimum, and often for 2-3

times this duration (Willner, 1997). There is substantial evidence that the duration of the stressor is critical; for example, in rats, decreased hippocampal volume was reported following unpredictable mild stress for 8 but not for 2 weeks (Luo et al., 2014). Accordingly, we considered it important to extend the duration of CSD (see Conclusions for further discussion of this key issue).

2.4 Gene x environment

Mouse psychosocial stressor studies are typically conducted with the inbred C57BL/6 strain. The contribution of differences in DNA sequence (i.e. polymorphisms) to inter-individual differences is therefore minimal. Variation will arise primarily due to environmental differences between litters (e.g. litter size and sex composition, maternal care) and within litters (e.g. social status, hierarchy stability), and their epigenetic consequences for individuals, it must be assumed. It is now widely accepted that gene x environment (GxE) interaction is the unit of epidemiological study required to increase understanding of the etiology of human stress-related disorders (e.g. Duncan and Keller, 2011; Pryce and Klaus, 2013). Given that transgenic animal models are typically constructed and produced in mice and back-crossed onto a C57BL/6 background, then there is considerable potential for GxE studies where the effects of specific genetic manipulations on susceptibility or resilience to chronic psychosocial stress (CPS) can be investigated (Pryce and Klaus, 2013). For example, the effects of knockout of the noradrenaline transporter (NET) gene were compared in control and CSD mice: in control mice, relative to wildtype, NET knockout led to reduced immobility in the forced swim test (an acute physical stressor) and increased interest in sucrose reward; furthermore, following CSD, NET knockout led to increased stress resilience in these same behavioral tests (Haenisch et al., 2009).

3. Psychosocial stressors in rats

Similar to the situation in mice, different social stressors are used in rats involving two or more animals in dyadic, group or colony situations (for reviews see e.g. Blanchard et al., 2001; Miczek et al., 2008; Hollis and Kabbaj, 2014; Slattery and Cryan, 2014; Koolhaas et al. this volume). These paradigms are proposed to be more relevant to the human situation than non-social stress paradigms e.g. repeated restraint stress (Slattery and Cryan, 2014). In experimental animals subjected to social-stressor paradigms, numerous brain, physiological and behavioral effects have been observed which resemble those in humans exposed to acute or chronic stressors.

3.1 A modified paradigm of chronic psychosocial stress

Fuchs and colleagues have developed a modified chronic social stress paradigm in rats based on the original resident-intruder paradigm (Rygula et al. 2005; 2006a; 2006b; 2008). The subjects were

experimentally naive adult male Wistar rats, housed individually in a colony room with a reversed 12h:12h light/dark cycle (lights on at 22:00 h) (see section 3.2). After arrival in the laboratory, the rats were habituated to maintenance conditions for 2 weeks and handled daily (control phase). Importantly, all experimental manipulations and behavioral tests were conducted during the dark phase of the light/dark cycle, specifically under dim red light and in the middle of the active (dark) period, between 10:00 and 16:00 h. Adult male Lister Hooded rats were used as residents. These animals were paired with sterilized females and housed in large plastic cages located in a separate room to the Wistar rats, but subjected to the same maintenance. Therefore, stress exposure was performed in a separate room (see section 3.3). Social defeat was induced as described previously by Tornatzky and Miczek (1994) and Koolhaas et al. (1997a). Briefly, before the start of the social defeat procedure, the female resident rats were removed from the cages. Each experimental male Wistar rat was transferred from its home cage and introduced into the resident's cage for 1 hour. Within 1-3 min, the intruder was attacked and defeated by the resident, as shown by freezing behavior and submissive posturing, whereupon the intruder and resident were separated. For the remainder of the hour, the intruder was kept in a small wire-mesh compartment within the resident's cage. Thus, the intruder animal was protected from direct physical contact, but remained in olfactory, visual and auditory contact with the resident. After this procedure, intruders were returned to their home cages. Intruders were subjected to such social defeat daily for 5 weeks. To avoid individual differences in the defeat intensity and to increase unpredictability, each day each intruder was confronted with a different resident according to a Latin square design. Each resident rat was used only once daily to maintain attack motivation. Control animals were handled daily throughout the entire experiment. Handling consisted of picking up each rat, transferring it to the experimental room and returning it to its home cage. The stressed rats were single housed after the defeat period (see section 3.3). For drug studies the test compounds were administered orally (drinking water or gavage) to mimic the clinical situation and to minimize stress from injections (see section 3.4). To obtain a realistic intervention, the treatment started when the stress-induced behavioral and endocrine changes were induced, after at least 7 days of the stressor, and the drugs were given daily whilst maintaining the psychosocial stressor. Finally, the therapeutic action of the drugs was monitored for a clinically relevant period of four weeks. Daily, throughout the experiment, each animal was weighed and in case of drug application via the drinking water, the fluid intake was monitored and the drug dose adjusted accordingly.

In rats submitted to five weeks of daily social defeat, reduced interest in gustatory reward, quite possibly related to the core symptom of reduced interest in human MDD, was induced (Rygula et al., 2005). Importantly, the stress-induced reduced reward-directed behavior was reversed in a time-dependent manner by daily administration of the selective serotonin reuptake inhibitor (SSRI)

citalopram for the clinically-relevant period of four weeks, thereby demonstrating the predictive validity of the model (Rygula et al., 2006a).Using the same paradigm, other stress-induced behavioral deficits were reversed by another SSRI, fluoxetine (Rygula et al., 2006b). For a further pharmacological validation of this chronic social stress paradigm, rats were subjected to 5 weeks of daily social defeat and a parallel treatment of four weeks with the selective noradrenaline reuptake inhibitor antidepressant reboxetine and the neuroleptic haloperidol; this polypharmacy approach was completed with the anxiolytic diazepam, administered acutely at the end of the stress period. Four weeks of oral treatment with reboxetine ameliorated the adverse effects of social stress and normalized behaviors related to motivation and reward sensitivity. The treatment with haloperidol worsened the adverse effects of chronic social stress, exerting effects on reward and motivation-related behaviors similar to those caused by stress. Diazepam reduced anxiety-related behaviors specifically. The effectiveness and selectivity of the treatment with the antidepressant reboxetine in ameliorating socially induced behavioral disturbances supports the validity of chronic social stress effects on reward-directed behavior as a MDD model in rats (Rygula et al., 2008). Below we provide further details on some important features of rat social stress paradigms and MDD models, as illustrated by the above studies.

3.2 Time matters

Rats, like mice, are nocturnal and thus generally quiescent during the light phase of the light-dark cycle and active during the dark phase. To identify and record as many behaviors and postures as possible, observations should be made when subjects are most active. For nocturnal animals therefore, determination of the effect of psychotropic drugs on natural action patterns of behavior should employ observations during the dark phase of the light-dark cycle. In most cases, for the convenience of the experimenter, this means that rats must be fully entrained to a reversed light-dark schedule (von Mayersbach, 1976; File and Hyde, 1978; Mitchell and Redfern, 2005). In line with the findings that the responses to stressors depend on the time of day at which the latter are applied (Dunn et al., 1972), we found that restraint stress in rats had a stronger impact on body weight and the weight of the adrenal glands when the animals were stressed during their active period. Moreover, we could show that the diurnal rhythm has a unique impact on the structural plasticity of pyramidal cells in prefrontal cortical areas of the brain and that stress interferes with this form of neuroplasticity (Perez-Cruz et al., 2009). The critical importance of the time of stressor exposure is emphasized by a recent study in mice: animals subjected to CSD during the active period developed more pathophysiological signs than those exposed during the inactive period (Bartlang et al., 2012). Whilst further work is required to provide functional insights into why the same stressors have a more pronounced negative outcome when applied during the active phase, one may speculate on

the following. Mechanistically, HPA axis activity peaks at the start of the active phase, i.e. in early evening in most rodent species and early morning in tree shrews, and humans. Both activity and rhythmicity of the HPA axis are controlled by efferents of the suprachiasmatic nucleus, the central pacemaker of the circadian system in mammals, to the paraventricular nucleus of the hypothalamus (Nicolaides et al., 2014). This nucleus is the key activator of the HPA axis during stress. Thus, it is likely that stressor exposures at distinct times of the light–dark cycle may generate different stress responses, resulting in mice stressed during the rest period exhibiting more adaptive responses and those stressed during the active period more maladaptive responses (Bartlang et al., 2012).

3.3 The impact of housing

The question of whether stressed animals influence non-stressed conspecifics is often neglected (Fuchs et al., 1987). Although this is of potential marked importance, many reports on stress experiments give no information on whether the experimental animals were completely separated from the rest of the colony, including the control group. It has been shown that odors from stressed rats act as signals to conspecifics, which then respond by overall changes in activity (Mackay-Sim and Liang, 1981a). These “alarm pheromones” are released rapidly and are probably derived from the body surface and urine of stressed rats (Mackay-Sim and Liang, 1981b). In addition, during aversive situations, rats produce ultrasounds that appear to play an important role as social signals and that affect the behavior of conspecifics (Sales, 1972). Such olfactory and auditory alarm signals may influence non-stressed conspecifics (controls) (Fuchs et al., 1987) and therefore stress experiments should be performed in separate rooms.

The housing conditions after social defeat appear to be crucial for the development of MDD-like symptoms in rats. Because an altered dopaminergic system is considered to be characteristic of stress-related MDD, we investigated the impact of individual and group housing on the temporal development of changes in dopamine transporter (DAT) binding in male rats after a single social defeat. We could show that single social defeat exposure induced a reduction of striatal DAT that developed gradually and lasted for at least 5 days after defeat. This effect was only evident if this stress situation was followed by social isolation. In rats that were returned to their familiar group after social defeat, the density of striatal DAT binding sites was not affected. This finding suggests that housing conditions are critical when investigating the central nervous system effects of social defeat in rats (Isovich et al., 2001).

3.4 Route of administration and drug monitoring

In most drug studies, test compounds are injected intraperitoneally (i.p.) or intravenously (i.v.). We decided to use oral application because this route of administration provides several advantages: (i)

it mimics the clinical situation where most patients take the drug orally; (ii) drugs taken orally produce metabolite concentrations that differ from those obtained after i.p. or i.v. administration, and (iii) it minimizes the stress effects of injections. Importantly, in pilot studies we determined the dose of the test compounds necessary to reach, in analogy to human patients, therapeutically relevant serum concentrations in the animals. Using this approach we found, for example, species differences in the metabolism of clomipramine in rats, tree shrews and humans (van Kampen et al., 2002). This finding demonstrates the need for monitoring the concentrations of circulating drugs and their pharmacologically active metabolites in animal studies. Otherwise, it cannot be excluded that sub- or supra-effective doses are being administered. To date this point has received little attention, but it should be considered when applying results from the treatment of experimental animals to the clinical situation.

Antidepressants are given to patients already exhibiting affective disorders and not prophylactically, of course. Accordingly, to mimic the situation of a therapeutic drug intervention, the treatment should start when the stress-induced behavioral and endocrine alterations are manifested, and not beforehand. Although therapeutic effects of most antidepressant drugs require 2-3 weeks to first appear, only a few animal studies have employed chronic administration of antidepressants over a clinically relevant time period (e.g. see Willner et al., 1992; Reul et al., 1993; 1994). In our studies, the drugs were administered daily in the morning before social defeat, while the psychosocial stress continued, and the therapeutic action of the test and reference compounds was followed across a clinically relevant time period of 4 weeks.

3.5 Psychosocial stress in females

Epidemiological studies demonstrate that the prevalence of affective disorders is at least twice as high in women as in men (Kessler, 2003). Surprisingly, however, very few preclinical studies have been conducted on female experimental animals, including in rat, and there is therefore a clear need to develop animal stress models in females for the study of stress-related disorder pathophysiology in women. Fuchs and others have observed that social defeat is generally an ineffective stressor in female rodents (Haller et al., 1999; Palanza, 2001). In female rats, a social-instability stressor paradigm was established, consisting of alternating periods of crowding and social isolation, together with rotation among social groups during the crowding phase. The paradigm has been shown to evoke acute stress responses (Haller et al., 1999). More recently we investigated whether 4 weeks of social instability induced a lasting change in physiological, brain and behavioral parameters in female rats (Herzog et al.,2009). Isolation or crowding by themselves are insufficient to induce a stress response in female rats, indicating that it is the instability in housing conditions that specifically leads to stress-induced changes (Benton and Brain, 1981; Brown and Grunberg, 1995). In the social

instability paradigm, female rats can be kept under chronic stress for weeks without habituation and ultimately they develop a MDD-relevant phenotype. At the physiological level, increased adrenal weight and plasma corticosterone levels indicate hyperactivity of the hypothalamus–pituitary–adrenal axis. Elevated plasma luteinizing hormone and disruption of the estrous cycle, together with increased serum prolactin levels, indicate dysregulation in the hypothalamus–pituitary–gonadal axis. Body temperature regulation was affected during the last week of stress, such that stressed females exhibited a lower reduction in body temperature during the rest phase compared with controls, i.e. a flattened basal temperature curve. Behaviorally, the chronically stressed female rats showed reduced sucrose preference and food intake (Herzog et al.,2009). Finally here, it should be noted that it was recently reported that social defeat can be induced in a female paradigm, by using older, lactating individuals as residents (Holly et al., 2012).

4. Psychosocial stressors in tree shrews

4.1 Exploiting territoriality

Over a number of years, evidence has accumulated that CPS in a non-rodent species, the male tree shrew (*Tupaia belangeri*), represents a natural and valid paradigm for studying the behavioral, endocrine, and neurobiological changes related to MDD. Phylogenetically, the tree shrew is placed together with Primates and Dermoptera within the clade Euarchonta (Kriegs *et al.* 2007). The close affinity between tree shrews and primates was further supported in a recent genome analysis (Fan et al. 2013). Compared with rodents, the tree shrew exhibits high homology to humans in terms of potential primary targets of psychotropic drugs e.g. glucocorticoid and mineralocorticoid receptors, CRH1 and CRH2 receptors, alpha$_{2A}$-adrenoceptor. For these receptors the tree shrew has 90-98% homology with the nucleotide sequences in human, compared with about 80% homology for rat and human. The degradation routes of psychotropic compounds are also more similar between tress shrew and human than rodent and human (see Fuchs 2005).

Tree shrews are diurnal mammals, widely distributed in South-East Asia. In their natural habitat males defend territories against intruding conspecifics (Kawamichi and Kawamichi, 1979). Originally developed by Raab (1971) and later adopted by von Holst (1977) and Fuchs, this pronounced territoriality has been utilized to establish a naturally occurring resident-intruder situation under experimental control in the laboratory. When living in visual and olfactory contact with a male conspecific by which it has been defeated, the subordinate tree shrew shows marked changes in behavior, physiology (e.g. endocrine function) and neurobiology. Subordinates lose body weight and develop reduced locomotor activity; their sleeping patterns are characterized by increased early-morning waking episodes, and their circadian rhythm is profoundly disturbed. Analysis of endocrine function in subordinates reveals consistently increased concentrations of

cortisol, increased adrenal gland weight, increased concentrations of noradrenaline indicating enhanced sympathetic activity, and reduced gonadal function (for review see e.g. Fuchs and Flügge, 2002; Czéh et al., 2016). By using a modified hole board we followed memory performance during > 20 weeks of alternating stress-free and stressful conditions. Despite normalized cortisol levels, significant memory deficits in experimental animals were observed even 10 weeks after the last stressful experience (Ohl and Fuchs, 1999). In a study with *in vivo* localized proton magnetic resonance spectroscopy (MRS) we found long lasting brain effects of psychosocial stress. In particular, the cerebral metabolite N-acetylaspartate (NAA) – found exclusively in neuronal tissue (Birken and Oldendorf, 1989) – was still elevated four weeks after the last stress exposure. In the same study we found that body weight had not returned to pre-stress levels after four weeks (Michaelis and Fuchs, unpublished data).

Since the distinct, stress-induced behavioral, physiological, and central nervous system alterations in subordinate tree shrews depend exclusively on the continuous visual presence - possibly promoting cognitive processing - of the dominant conspecific (Raab and Storz, 1976; Raab and Ostwald, 1980), this paradigm was perhaps the first to be termed psychosocial stress. Across a large number of experiments, about 90% of tree shrew intruder males exhibited stressor susceptibility with respect to markers such as loss of body weight and reduced locomotion, with about 10% being resilient.

4.2 Predictive validity for depression

To investigate whether tree shrew psychosocial stress-induced neurobehavioral dysfunction exhibits predictive validity as a MDD model, Fuchs and colleagues treated subordinates with established and potential antidepressants including clomipramine, fluoxetine, tianeptine, agomelatine, different NK1 receptor antagonists, and the synthetic neurosteroid 3β-methoxypregnenolone. It is important to note that: (i) we determined and used the appropriate dose of the antidepressants necessary to reach therapeutically relevant serum concentrations; (ii) the daily oral treatment commenced only when the stress-induced behavioral and endocrine changes were clearly established; (iii) the psychosocial stress situation was continued during the treatment; (iv) the therapeutic action of the drug was assessed for the clinically appropriate period of time of four weeks, and; (v) the action of the antidepressant had a time-dependent onset, in some cases requiring several weeks of chronic treatment to reach efficacy. All drugs were given in the morning before stress exposure with the exception of agomelatine which was applied shortly before the onset of the resting period. Using this approach, in subordinate animals we observed a time-dependent normalization of endocrine, behavioral and central nervous parameters (Czéh et al., 2001; Czéh et al., 2005, Fuchs et al., 1996; van der Hart et al., 2002; van der Hart et al., 2005; Schmelting et al., 2014; Parésys et al, 2015). In

contrast, the anxiolytic diazepam was ineffective in this experimental setting (van Kampen et al., 2000). Our findings with clomipramine were recently confirmed, showing that changes analogous to core symptoms of MDD could be reversed in subordinate tree shrews by chronic treatment with this established tricyclic antidepressant (Wang et al., 2013). Based on these findings the CPS paradigm in tree shrews can be regarded as providing a 'homologous model' of MDD: it mimics several aspects of the human disease in the animal; the state of the animal is induced by similar stimuli that cause the condition in humans; and pharmacotherapy that is efficacious in the human illness is effective in the model. The advantage of a homologous model is that it can probably contribute to the understanding of the brain biochemistry of MDD and it might also lead to the development of effective novel drugs for treatment of the illness.

(TABLE 1 ABOUT HERE)

5. Conclusions

5.1 Etiological validity of adulthood chronic psychosocial stressors

The evidence that human depression is often preceded by a (reported) period of adulthood chronic psychosocial stress has resulted in animal studies in which species-relevant psychosocial stressors have been developed and their neurobehavioral effects investigated. Here we describe three such paradigms, one each for the mouse, rat and tree shrew. The major characteristics of the paradigms are presented and compared in Table 1. As explained above, each is based on the resident-intruder paradigm, and involves the intruder experiencing threat and attack that is uncontrollable in that submissive behavior does not result in cessation of attack. One variable on which the paradigms differ considerably is the duration of the stressor: 15 days in mouse chronic social defeat, 5 weeks in rat chronic psychosocial stress and 4-5 weeks in tree shrew chronic psychosocial stress. As noted above, the interval between stressor onset and depression onset is typically 3-6 months, strongly suggesting that the longevity of the stress state is of major importance to the pathophysiological mechanism(s) underlying depression. With respect to the observed chronicity of the effects of the manipulation, CSD mice exhibit generalized helplessness in terms of impaired two-way active avoidance at 15 days after the last day of attack (Azzinnari et al., 2014), CSS rats show structural changes in the brain that may last for several weeks (Kole et al., 2004), and CPS tree shrews exhibit brain biochemical changes at 4 weeks and memory deficits at 10 weeks (see above). At least for CSD mice, therefore, it needs to be investigated whether the effects persist beyond 15 days, and are thereby commensurate with the study of pharmacological reversal of CSD effects and comparison of fast- versus slow-acting antidepressant classes. That effect longevity is sufficient for this in rat CSS and tree shrew CPS has been demonstrated, as described above. A serious limitation of each of these

stressor paradigms is that, whilst they utilize the species-typical traits of territoriality and social hierarchy based on aggression, these traits are restricted to or at least much more pronounced in males than females, thereby meaning that females cannot be studied in the paradigms. However, in mice it has been demonstrated that when females are used in a modified CSD protocol, where they are exposed to the resident attacking a male intruder, then they do indeed develop similar behavioral changes to those that occur in males in the typical CSD paradigm (Avgustinovich and Kovalenko, 2010).

Comparing the species and respective paradigms with each other, for mouse CSD a relative advantage is that the availability of transgenic models allow for the extensive study of gene x environment (GxE) interaction effects and therefore the modeling of the susceptibility/resilience effects of human polymorphisms, whereas a relative disadvantage is the limited chronicity of the stressor in the current paradigm. For rat CSS, a relative advantage is the extended period of stressor chronicity, and a relative disadvantage is that there are periods of resident-intruder separation meaning that there are periods without stressor exposure. For tree shrew CPS, a relative advantage is the high human homology at DNA and protein levels, which becomes particularly relevant at the compound screening stage of studies, and a relative disadvantage is that few laboratories have the necessary capacity and expertise to apply this stressor.

5.2 Inter-individual differences in responding to chronic psychosocial stressors

Variation in human populations in terms of gene polymorphisms, epigenetic markers and developmental life-history experiences means that responsiveness to adulthood chronic psychosocial stressors will exhibit marked inter-individual differences. Some individuals will be susceptible to modest CPS in terms of developing depression and other individuals will be resilient to extreme CPS in terms of not developing a depression. Depression 12-month prevalence is about 7-10 % (Wittchen et al., 2011). It might be argued, therefore, that in a valid animal model of stress-related depression, only a minority of animals will exhibit depression-relevant effects. In the case of species exhibiting low genetic and epigenetic variability, which is particularly the case for the inbred C57BL/6 mouse in the present study species, it will be difficult to establish such a model because the majority of mice will be either susceptible or unsusceptible (resilient). In the case of the majority of chronic psychosocial stressors studied, however, and certainly the three presented here, the majority of subjects exhibit behavioral, physiological and neurobiological effects that are in a depression-relevant direction relative to the average control condition (Table 1, Extent of effects vs controls). Whilst a statistically significant difference between the entire stress and control cohorts is typically used to define a depression-relevant effect, it might be preferable to focus on the most stress-reactive mice e.g. most-reactive 25% or 33%. Of course, the parameter on which to measure and

stratify the stressed subjects then becomes absolutely critical, and it is also essential to assess whether subjects that are highly stress reactive on one readout are similarly reactive on another readout.

5.3 Concluding remarks

Adulthood chronic psychosocial stressors impact on human behavior, physiology and brain structure-function to an extent that they constitute a major risk factor for depression and other affective disorders. That this relationship is not restricted to humans, and that it is indeed causal, is demonstrated by species-relevant manipulations leading to similar effects in other species. Whilst stressor features such as threat and uncontrollability appear to be consistent across species, there are certainly likely to be human versus non-human differences, such as whether or not the stressor needs to be continuously present physically and human-specific cognitive processes such as attribution of whether "the environment is" or "I am" to blame for "my" stressed state. With the paradigms we present here for mouse, rat and tree shrew, it has been possible to demonstrate pharmacological effects of existing antidepressant drugs. That more efficacious drugs are required is clear, and we are also optimistic that animal models based on adulthood chronic psychosocial stress will be important in enabling such drugs to be discovered and developed. As we have noted, the available species and models bring advantages and disadvantages with them, and so it is careful application of specific models for specific research questions that is likely to yield the highest probability of novel, efficacious therapeutics in this research field.

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Table 1. Comparison of some important parameters and outcomes in adult chronic psychosocial stressor paradigms in mouse, rat and tree shrew

	Mouse	Rat	Tree shrew
Paradigm	Chronic social defeat (CSD), refined (Azzinnari et al., 2014)	Chronic social stress (CSS), refined (Rygula et al., 2005)	Chronic psychosocial stress (CPS) (Fuchs and Flügge, 2002)
Procedure	Resident-intruder + continuous distal exposure	Resident-intruder + 1-hr distal exposure	Resident-intruder + continuous distal exposure
Intruder	C57BL/6 male	Wistar male	Subordinate tree shrew male
Resident	CD-1 male, ex-breeder, teeth trimmed	Lister Hooded male, breeder	Dominant tree shrew male
Duration	15 days, 1 min attack per day	5 weeks, 1-3 min attack + 1-hr distal exposure per day	4 – 5 weeks Direct contact max. 1-hr per day
Chronicity of effects	At least 2 weeks beyond stressor (Behavior)	Several weeks (Brain) (Kole et al., 2004)	At least 4 weeks (Brain), some effects at 10 weeks after stressor onset (Behavior)
Limited to males	Yes, but modified protocol for females (Avgustinovich & Kovalenko, 2010)	Yes, but modified protocol for female Long-Evans rats (Holly et al., 2012)	Yes
Extent of effects vs controls	Some overlap, approx. 70% of CSD without overlap to controls	Some overlap, approx. 90% of CSS without overlap to controls	Some overlap, approx. 90% CPS without overlap to controls
Relative advantages	Transgenic models for GxE study	Extended period of stressor chronicity	High human homology
Relative disadvantages	Limited stressor chronicity	Periods without stressor exposure	Few laboratories with capacity and expertise